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STUDY OF VIABILITY OF MICROORGANISMS IN SIMULATED SPACE

Final Report
April 21, 1963 through May 31, 1964

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

PAGE

SUMMARY

33099

Viability studies were conducted on selected microorganisms subjected to ultrahigh vacuum (UHV), in the 10^{-9} torr range, and exposed to each of the following: (1) ultraviolet radiation, (2) gamma radiation, and (3) elevated temperatures.

Among spores of four test organisms subjected to UHV and ultraviolet radiation, A. niger proved the most resistant; B. megaterium, B. subtilis and B. stearothermophilus proved about equally less resistant. All four spores were less radio-resistant when UHV-dried than when air-dried.

Among spores of four test organisms subjected to UHV and gamma radiation, B. megaterium proved the most resistant; A. niger proved least resistant; B. subtilis and C. sporogenes were of intermediate resistivity. All four spores were less radio-resistant when UHV-dried than when wet.

Microbial flora of dried desert soil withstood 200°C simultaneously with UHV for five days. Soil isolates showed considerably less resistivity, however, thus confirming that soil serves as a protective agent against thermal destruction.

Three vegetative microorganisms subjected simultaneously to elevated temperatures and UHV showed a rapid decrease in survival as temperatures rose above approximately 50°C. S. aureus was the most resistant organism, while culture 248 (an extremely radiation-resistant coccus isolated in the laboratory) was the least resistant. Survival curves of both these organisms began leveling off in the vicinity of 70°C. S. faecalis was of intermediate resistivity.

INTRODUCTION

This final summary report describes research conducted for the National Aeronautics and Space Administration under Contract NASw-773, "Study of Viability of Microorganisms in Simulated Space." It covers the period April 21, 1963 through May 31, 1964. The studies reported were a continuation of those conducted under Contract NASr-41. The program was a joint effort of National Research Corporation, through its Space Vacuum Laboratory, and the Massachusetts Institute of Technology, through the Microbiological Research Laboratory of the Department of Nutrition and Food Science.

The following studies are herein reported:

(1) Resistivity of four test organisms to ultraviolet light in combination with ultrahigh vacuum.

(2) Resistivity of four test organisms to gamma radiation in combination with ultrahigh vacuum.

(3) Resistivity of soil microorganisms and of three test vegetative cells to elevated temperatures in combination with ultrahigh vacuum.

Work performed in the last quarter (February 1, 1964 to April 30, 1964) is discussed in greater detail than that already described in the first three quarterly reports.

I. RESISTIVITY OF MICROORGANISMS TO ULTRAHIGH VACUUM (UHV) AND ULTRAVIOLET (UV) RADIATION

The following four test organisms were used in this study: Bacillus megaterium, Bacillus subtilis var niger, Bacillus stearothermophilus and Aspergillus niger.

Experimental Procedure:

Preliminary experiments were conducted which demonstrated that microorganisms were protected from UV when supported on glass fibre filters due to their grossly irregular surfaces. Membrane filters (made by the Millipore Corp.) although fibrous in nature, demonstrated no such protection and were therefore subsequently employed in the experiments.

The spores, thus supported, were positioned within a vacuum chamber (which was kept at 2.0×10^{-9} torr) and exposed to a UV lamp. Replicate filters were placed inside glass tubes in the vacuum chamber to protect the organisms from UV while allowing their exposure to vacuum. The latter organisms thus served as controls. Further procedural details can be found in the October 31, 1963 Quarterly Report.

Apparatus:

Commercial UV lamps have extremely high intensity outputs. This condition, coupled with the close proximity between lamp and sample necessitated by vacuum chamber dimensions, causes unusably high microorganism dose rates. A lamp-current controlling device was therefore developed to reduce this intensity. As a result, a dose of 500 microwatts was deliverable over two minutes instead of several seconds, as occurred previously. Precise dose administrations were thus attainable. Troublesome intensity fluctuations were also reduced.

Fig. 1 shows the UV and UHV apparatus employed. A General Electric-type G15 Ta germicidal lamp was mounted centrally on a 20-cm diameter stainless steel cylindrical framework (the wire mesh screen around the lamp assisted in UV intensity reduction). Equipment was constructed for easy removal from the vacuum chamber to allow test specimen manipulation without changing lamp and cylindrical frame positioning. The lamp was aged to achieve a stabilized output and was calibrated by simulating sample exposures in air, nitrogen and vacuum. A Westinghouse UV meter with peak sensitivity at 2537Å was used for intensity measurements.

Pins were inserted in the frame to support 36 filters (which were 47 mm in diameter), each positioned 10 cm from the lamp. This unit was set on a tripod in the bell jar portion of the vacuum chamber. Spores on the filters were thus irradiated, both at atmospheric pressure and in ultrahigh vacuum.

Filters containing the controls were placed on a wire tree inside sterile pyrex test tubes. A sterile pyrex beaker supported on a wire covered the end of each tube. Controls were thus shielded from UV without impeding vacuum pumpdown. Three or four such tubes were positioned vertically in the base of the vacuum chamber.

Results:

Experimental results are presented in Table 1. Column A gives the percentage survival of spores exposed to UV after being air-dried but before being subjected to UHV. Column B gives the percentage survival of spores exposed to UV after being stored for 7 days over dessicant but before subjected to UHV. Column C gives the percentage survival of spores exposed to UV after being subjected to 7 days of UHV. Column D compares the number of viable spores prior to 7 days of dessication with the number remaining subsequently.

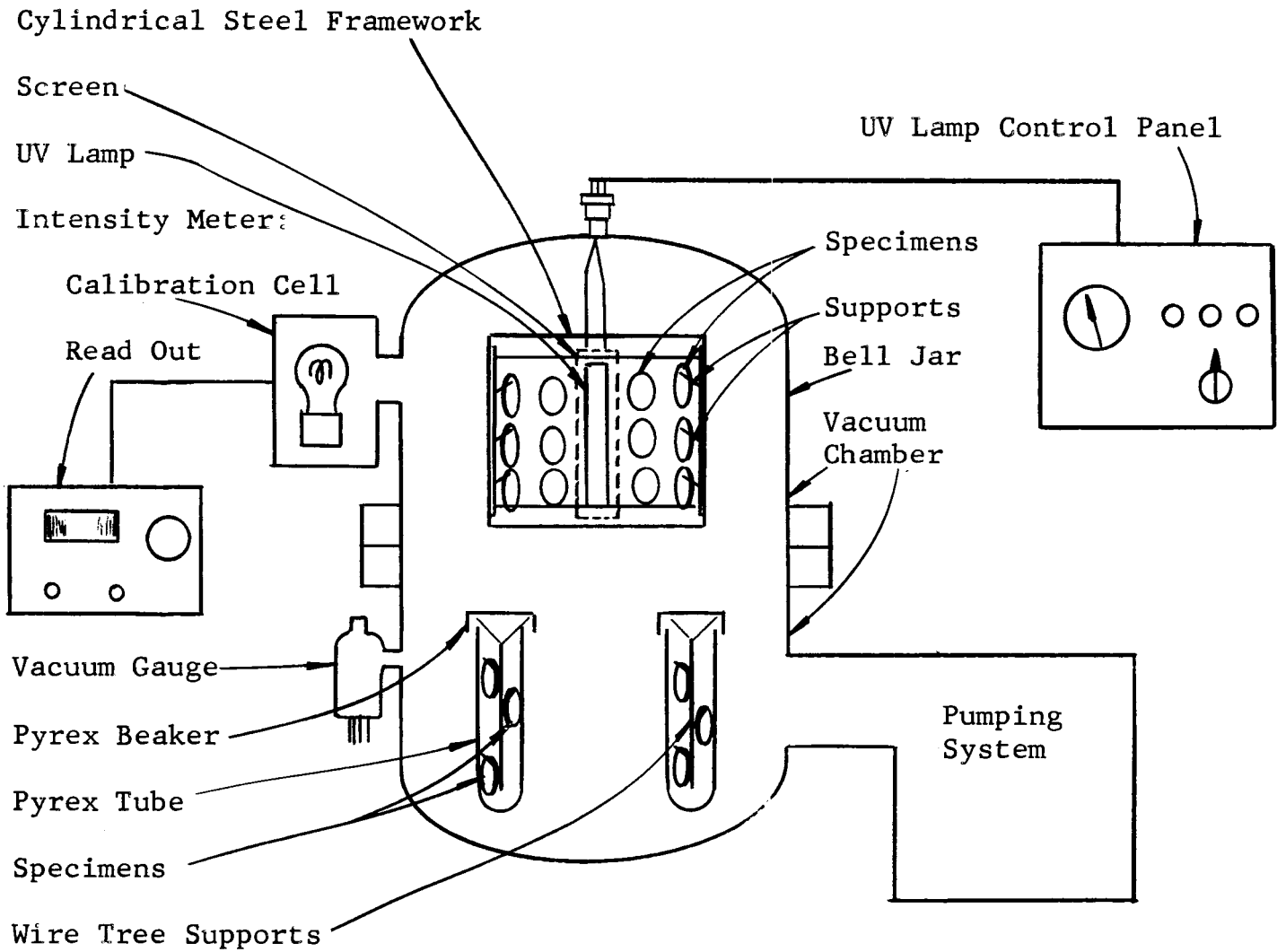

FIGURE 1

ULTRAHIGH VACUUM ULTRAVIOLET APPARATUS

TABLE 1

PER CENT SURVIVAL OF SPORES OF FOUR ORGANISMS AFTER ULTRAVIOLET IRRADIATION IN ULTRAHIGH VACUUM AND AT ATMOSPHERIC PRESSURE

Organism	Dose MW-sec / cm^2	A UVI(100)/DCI	B UVF(100)/DCF	C VUV(100)/V	D DCI(100)/DCF
B. megaterium	500	81	94	1.3	100
	750	83	100	0.06	117
	1,000	42	58	0.009	109
	10,000	0.33	0.49	0.002	104
B. subtilis var niger	500	55	42	5.0	100
	750	19	15	0.39	83
	1,000	6.5	9.5	1.2	105
	2,500	0.33	0.31	0.04	94
	10,000	0.03	0.02	0.001	1.23
B. stearothermophilis	500	38	33	1.0	104
	750	14	15	0.05	82
	1,000	1.0	5.9	0.23	97
	2,500	0.17	0.26	0.03	90
	5,000	0.08	0.08	0.02	76
A. niger	1,000	-	83.0	83.0	-

V: vacuum UV: ultraviolet DC: desiccator control

I: initial F: final

Of the four test organisms, A. niger was the most resistant, while the remaining three showed resistivity comparable with one another. Spores exposed to UV in the vacuum-dried state (Column C) were less resistant than those exposed to UV in the air-dried state (Columns A and B). There was little difference in resistivity between spores air-dried for 7 days (Column B) or for one day (Column A).

The reason for the greater susceptibility of the vacuum-dried spores to UV is not evident. However, two hypotheses are possible, if it is assumed that the DNA of the cell is the main target for UV damage and that indirect effects do not exist as a result of the organism's dessicated state. One hypothesis is that upon drying, the more susceptible portions of the DNA molecules are somehow exposed to UV. The other possibility is that, in some manner, repair mechanisms are interfered with, either through damage by long-lived and stable radicals or by spatial disruption.

II. RESISTIVITY OF MICROORGANISMS TO ULTRAHIGH VACUUM (UHV) AND GAMMA RADIATION

The following four test organisms were used in this study: B. megaterium, B. subtilis var niger, A. niger and C. sporogenes. Radiosensitivity was determined for spores that were wet, air-dried and UHV-dried. Irradiation was conducted, both at atmospheric pressure and in vacuum. The results are presented in Fig.'s 2 through 5.

Two significant effects are to be noted. One is an oxygen effect; all four organisms showed an increased radio sensitivity in vacuum when oxygen was present. The other effect is the increased radiosensitivity caused by UHV drying (at 10^{-9} torr), as was noted above for UV. This can be seen in Fig.'s 2 through 5 by comparing wet spores irradiated in the presence of air with UHV-dried spores irradiated in the presence of air. This UHV drying effect has also been reported by Tallentire (Nature: 182: 1024, 1958). (While he limited his investigation to B. megaterium, it appears to be a general phenomenon.)

It is interesting to note that spores of B. subtilis var niger and of A. niger showed less radiosensitivity when UHV-dried and irradiated without air present than when wet and irradiated in air, while the reverse was true for the remaining two organisms. Also to be noted is that B. megaterium spores generally displayed the least radiosensitivity and A. niger the greatest.

Further discussion of these results and of the experimental procedures employed can be found in the January 31, 1964 Quarterly Report.

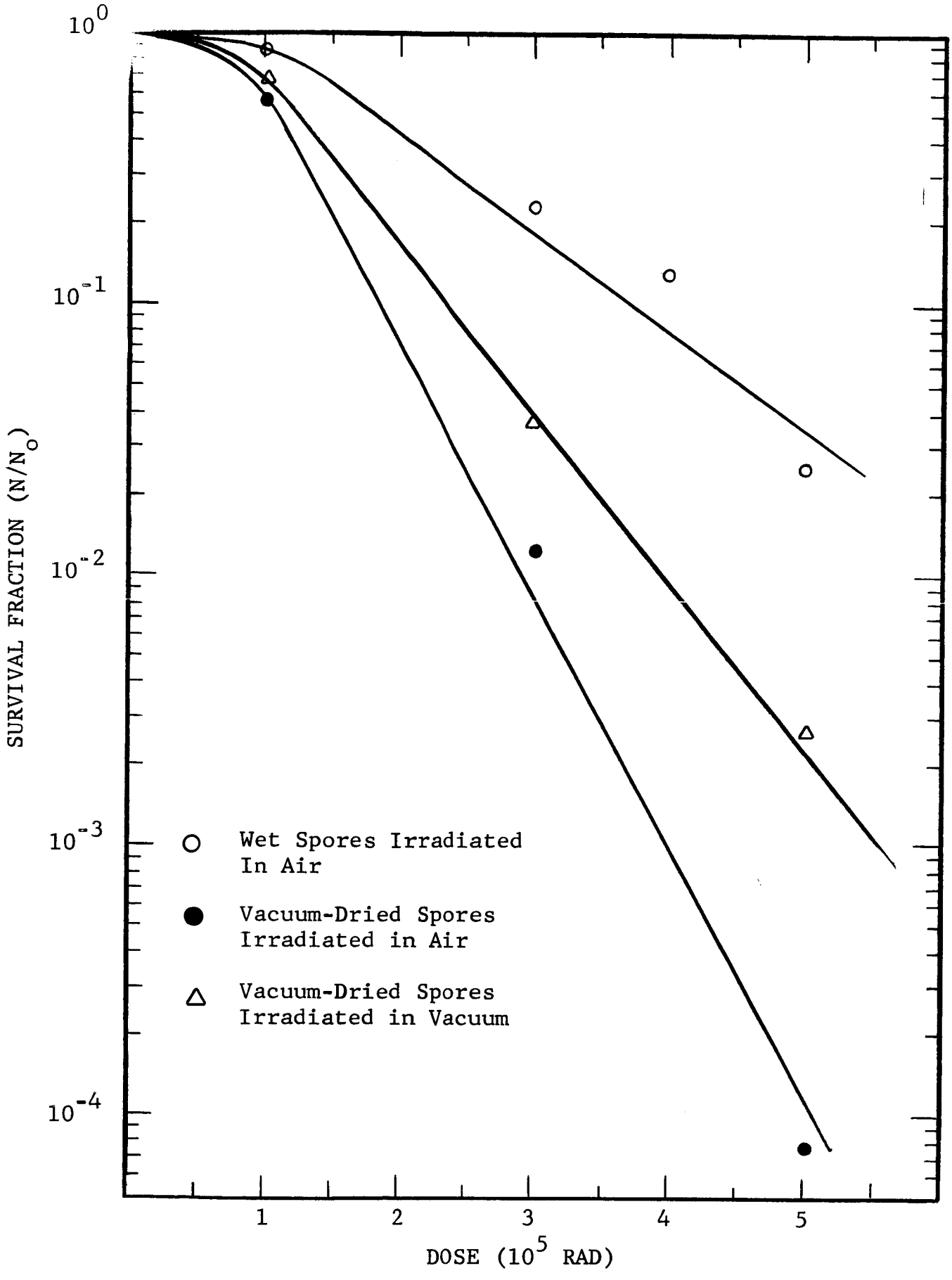

FIGURE 2

RADIORESISTIVITY OF BACILLUS MEGATERIUM SPORES TO GAMMA RADIATION

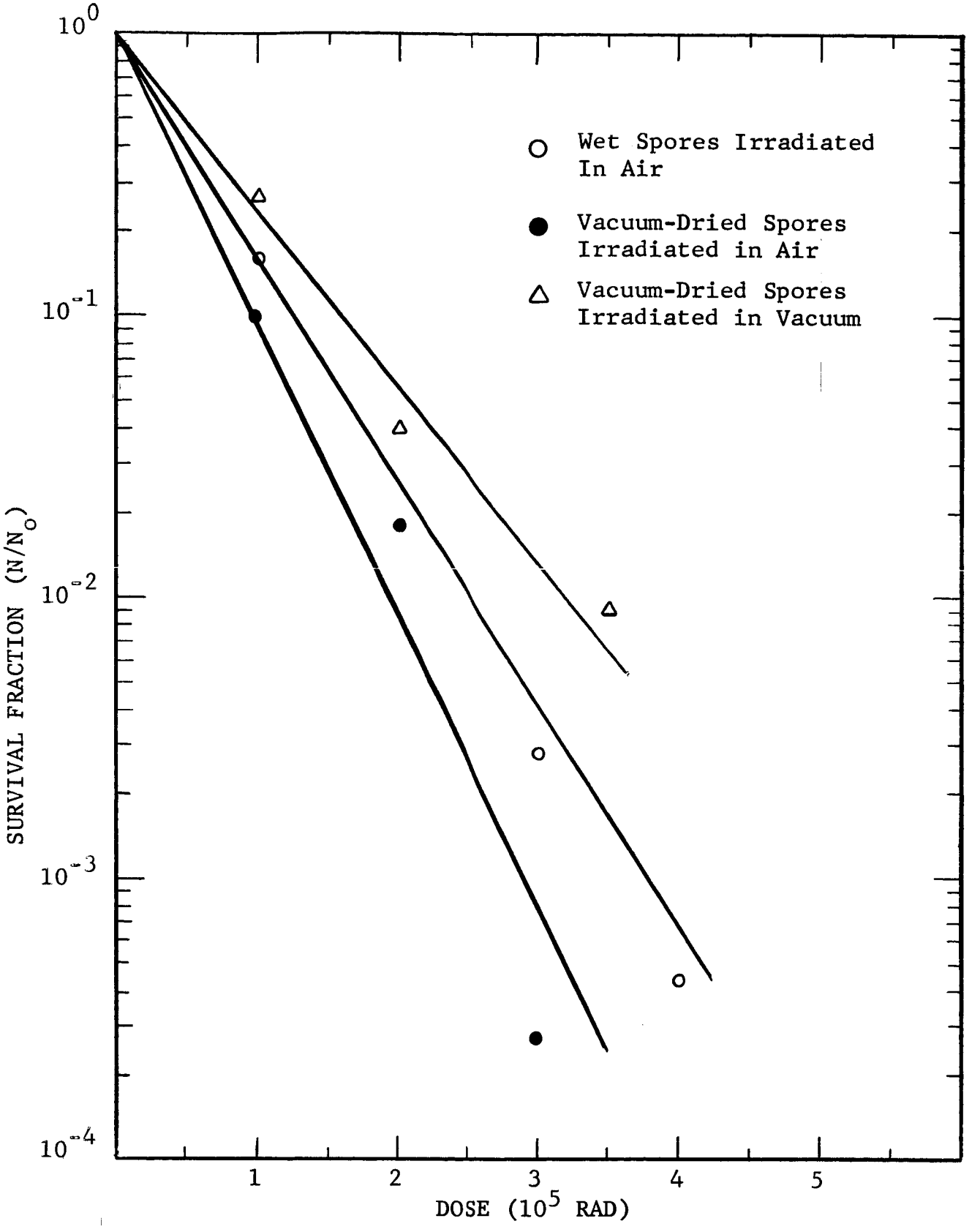

FIGURE 3

RADIORESISTIVITY OF BACILLUS SUBTILIS VAR. NIGER SPORES TO GAMMA RADIATION

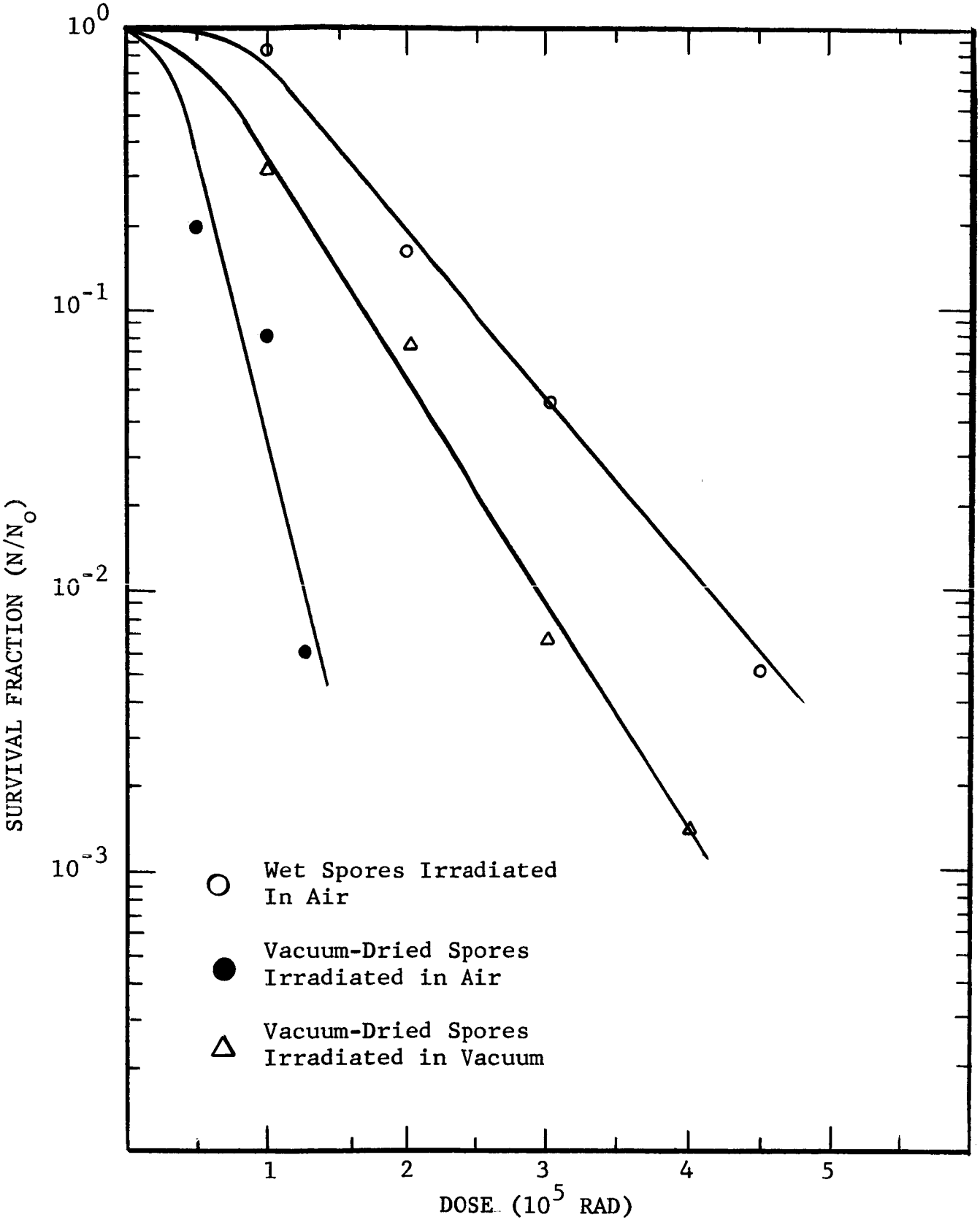

FIGURE 4

RADIORESISTIVITY OF CLOSTRIDIUM SPOROGENES SPORES TO GAMMA RADIATION

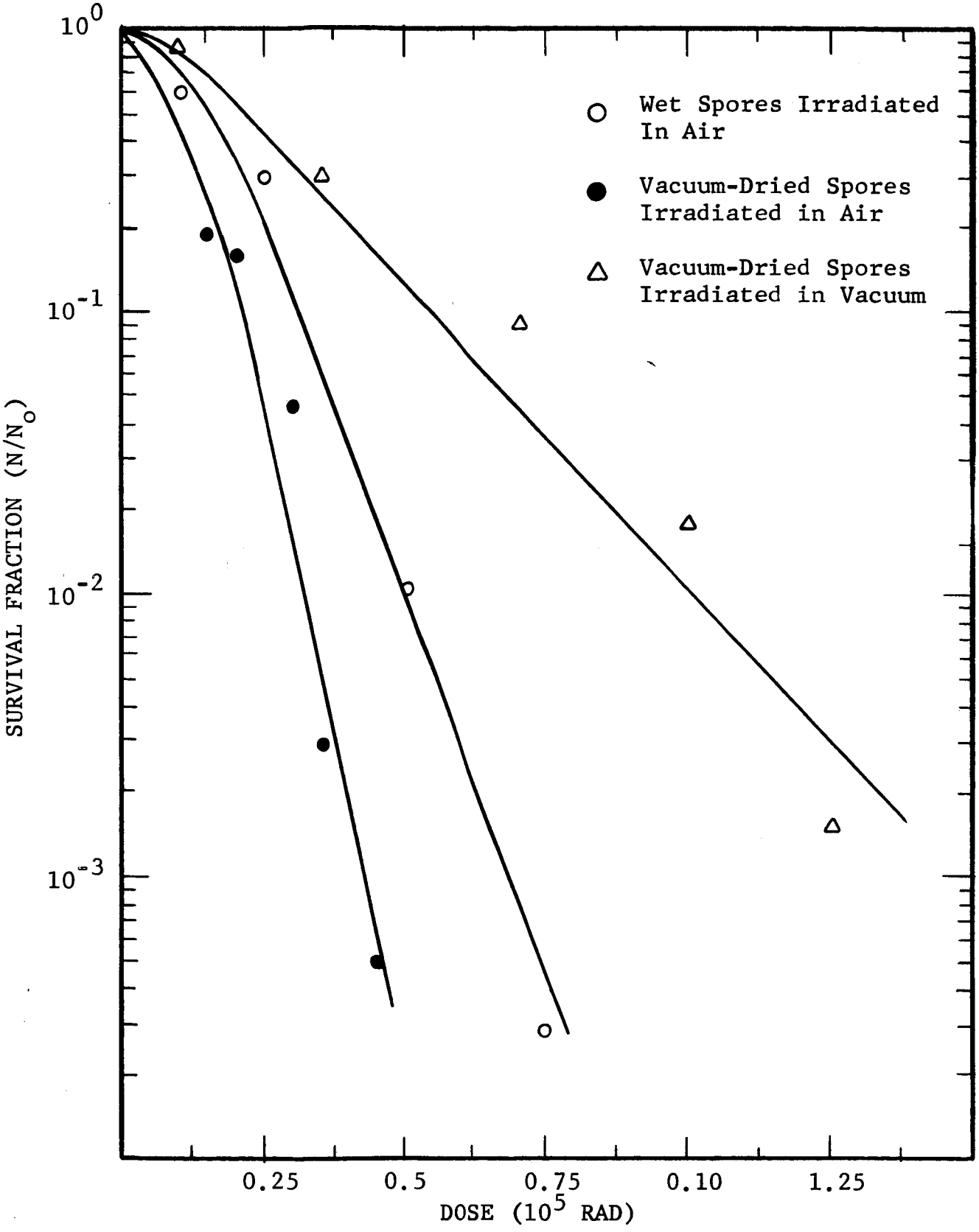

FIGURE 5

RADIORESISTIVITY OF ASPERGILLUS NIGER SPORES TO GAMMA RADIATION

III. RESISTIVITY OF MICROORGANISMS TO ULTRAHIGH VACUUM (UHV) AND ELEVATED TEMPERATURES.

A. Soil Microorganisms

It has been established that microbial flora in dried desert soils will survive 200°C for 4 to 5 days under ultrahigh vacuum (in the 10^{-9} torr range); both aerobes and anaerobes were recovered after this treatment. The main lethal deterrent appeared to be the protection afforded by (1) the soil itself, and (2) the physiological state of the organisms.

In other experiments, survivors of 200°C were isolated on filters and resubjected to ultrahigh vacuum. Two out of 70 cultures (35 aerobic and 35 anaerobic) survived only about 120°C. Such a qualitative test is inadequate to designate the cause of thermal sensitivity in soils, but it verifies that the two protective factors stated above were not re-established on the filters and thus confirmed that soil is inherently protective against thermal destruction (in ultrahigh vacuum).

Further details of this work are contained in the Quarterly Reports of July 31, 1963 and of October 31, 1963.

B. Vegetative Test Organisms

Three vegetative organisms were selected for this study: Streptococcus faecalis 10C1, Staphylococcus aureus, and an extremely radiation-resistant coccus No. 248, which was isolated in the laboratory.

Experimental Procedure:

S. faecalis and S. aureus were grown as stationary cultures for 22 hours at 37°C in tyrptone-glucose extract broth (difco), whereas 248 were grown as a 36-hour shake culture at 30°C in plate count broth supplemented with 0.5% N-Z case (Sheffield

Chemical Co.). The organisms were enumerated on agar of the same composition as the propagation broth; the incubation period was 5 to 12 days.

Appropriate dilutions of the stationary-phase cells were pipetted onto glass-fiber filter discs and dried overnight in a desiccator over silica-gel. The filter discs were then placed in a vacuum chamber and heated. Prescribed temperatures and ultrahigh vacuum (in the 10^{-9} torr range) were maintained for 5 days. After this time, the filter discs were blended in chilled aqueous diluent containing 0.1% trypticase (with a pH of 7.0) and serially diluted for quantitation in diluent of the same composition.

Apparatus:

A refrigerated vacuum system capable of attaining a pressure of 10^{-10} torr at ambient temperature and a specially designed radiant heater were employed. This apparatus is shown in Fig. 6.

The heater consisted of a tantalum wire coil supported on a frame by ceramic insulators. A stainless steel reflector surrounded the frame. The heater assembly was 10 1/2 inches in diameter and 11 1/2 inches in height. Two circular, stainless steel sample trays, each 8 1/2 inches in diameter, were located in an area whose temperature was regulated to within $\pm$ 1.0°C by a pyro-vane temperature indicating controller. The sample area temperature was monitored by several chromel-alumel thermocouples. Temperatures as high as 180°C $\pm$ 1.0°C were attainable while simultaneously maintaining a vacuum of 10^{-9} torr.

Results and Discussion:

Experimental results are presented in Table 2 and Fig. 7. As shown in Table 2, the initial number of organisms of each type was kept between 10^7 and 10^8 cells per filter. Also shown is

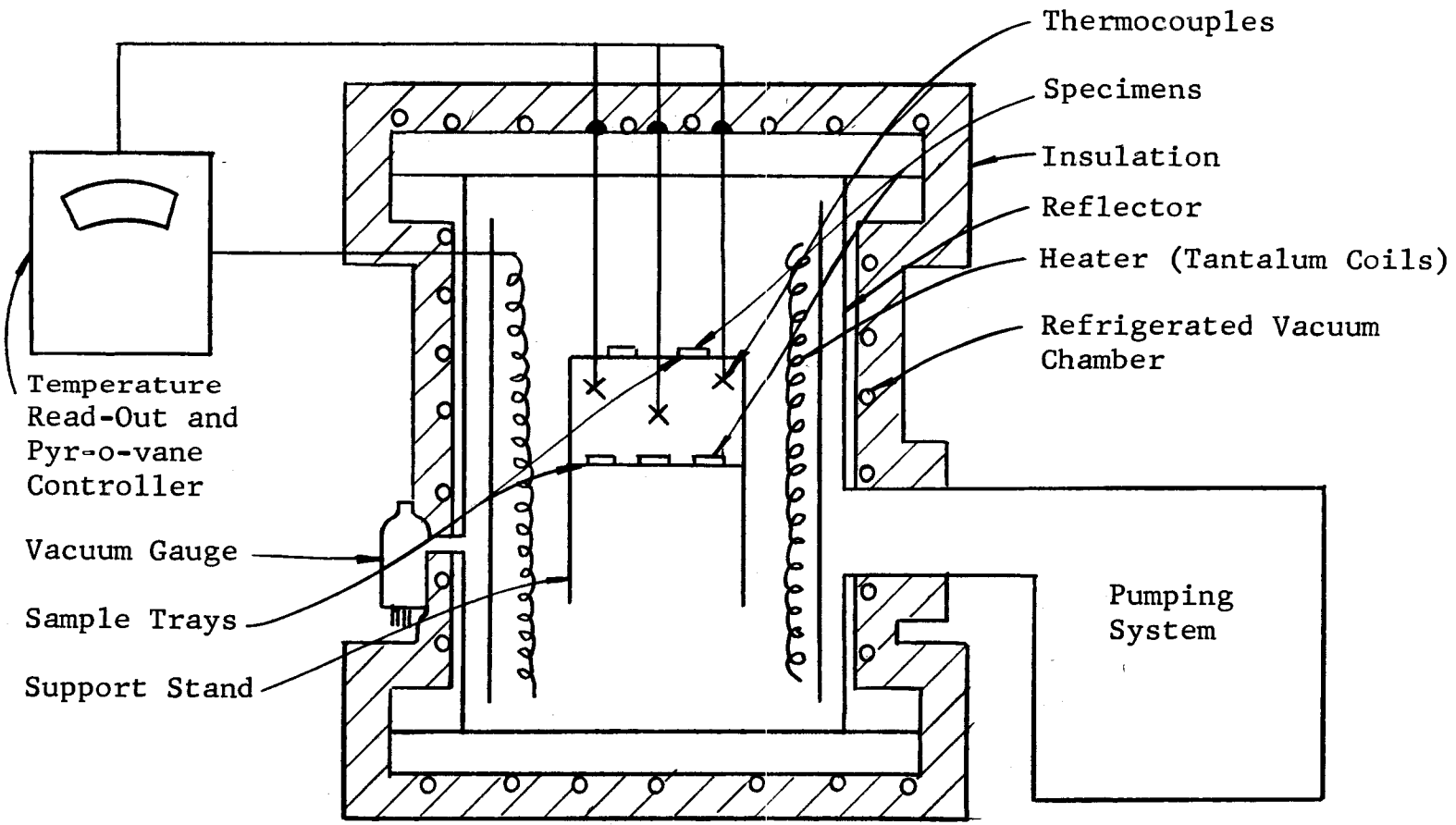

FIGURE 6

ULTRAHIGH VACUUM ELEVATED TEMPERATURE APPARATUS

TABLE 2

SURVIVAL OF VEGETATIVE CELLS AT VARIOUS TEMPERATURES WHILE UNDER ULTRAHIGH VACUUM

	Temp. C^o	N	N_o	N/N_o
Staphylococcus aureus	4.4	$1.0\ 10^6$	$26\ 10^7$	$4.0\ 10^{-2}$
	40	$2.3\ 10^6$	$3.0\ 10^7$	$7.6\ 10^{-2}$
	60	$2.6\ 10^5$	$3.0\ 10^7$	$8.6\ 10^{-3}$
	65	$3.3\ 10^3$	$3.1\ 10^7$	$1.0\ 10^{-4}$
	75	$9.0\ 10^2$	$3.1\ 10^7$	$3.0\ 10^{-5}$
Streptococcus faecalis	4.4	$1.4\ 10^6$	$1.3\ 10^7$	$1.0\ 10^{-1}$
	20	$1.4\ 10^6$	$1.3\ 10^7$	$1.0\ 10^{-1}$
	40	$1.4\ 10^6$	$1.3\ 10^7$	$1.0\ 10^{-1}$
	40	$2.0\ 10^6$	$1.5\ 10^7$	$1.3\ 10^{-1}$
	60	$2.3\ 10^5$	$1.5\ 10^7$	$1.4\ 10^{-2}$
	65	$3.6\ 10^4$	$1.1\ 10^7$	$3.2\ 10^{-3}$
	70	$2.0\ 10^1$	$1.6\ 10^7$	$1.3\ 10^{-6}$
	75	No Survival		
Strain 248	4.4	$3.1\ 10^6$	$1.5\ 10^7$	$2.0\ 10^{-1}$
	20	$4.7\ 10^6$	$1.5\ 10^7$	$3.1\ 10^{-1}$
	40	$1.7\ 10^6$	$1.5\ 10^7$	$1.1\ 10^{-1}$
	60	$6.6\ 10^4$	$8.1\ 10^7$	$8.0\ 10^{-4}$
	70	$4.0\ 10^1$	$7.2\ 10^7$	$5.5\ 10^{-8}$
	80	$2.0\ 10^1$	$7.2\ 10^7$	$2.8\ 10^{-8}$

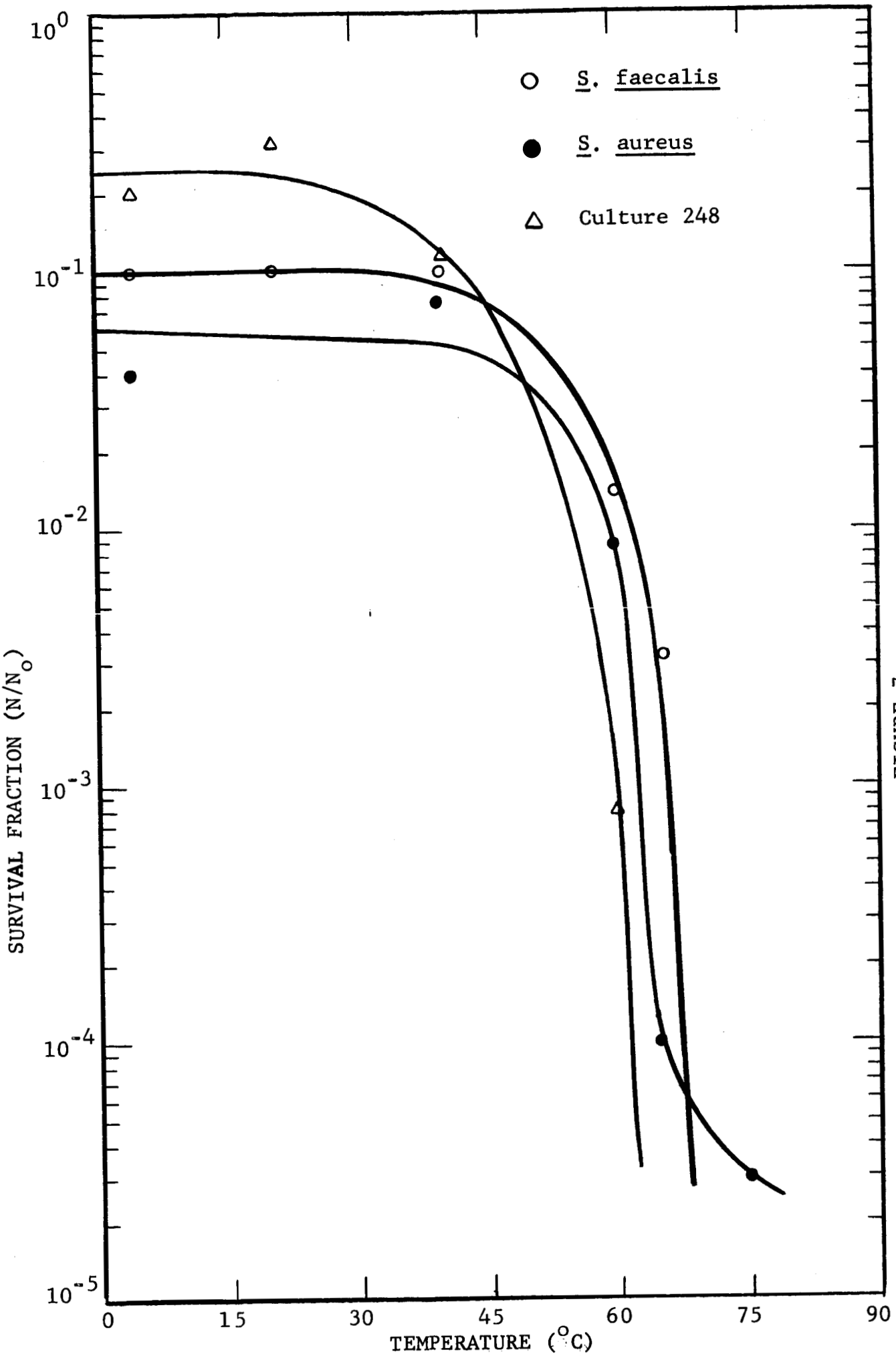

FIGURE 7

SURVIVAL OF VEGETATIVE CELLS IN ULTRAHIGH VACUUM TO ELEVATED TEMPERATURES

that the population as a function of temperature remained stable from 0°C to approximately 50°C, after which a rapid decrease occurred. The latter can also be seen in Fig. 7. Also to be noted is that culture 248 manifested survivors or "tailing off" in the range of 60°C to 80°C. (This is shown in Table 2 but not in Fig. 7.) S. aureus was the most resistant of the cells examined; it survived 75°C with only a 3 1/2 log cycle decrease, after which it too gave indications of a "tailing off" effect. S. faecalis was of intermediate resistivity, being more resistant than S. aureus at lower temperatures and then dying much more rapidly than culture 248 at higher temperatures (i.e., above about 65°C.)

The 50°C transition temperature noted above is surprisingly close to that noted in previous studies with spores, where 60°C was the critical temperature. The nature of the survival data indicates that rapid cell constituent destruction occurs near this temperature. Since this appears so for all three organisms, the lethal effect may be due to some general reaction(s).